Remarks:

Claims 1, 3-6, 9, 29, 61, 62, 69, 70, 72, 73, and 86-107 are currently pending in this application. In this response, previously pending Claims 7,8, and 12-17 are cancelled without prejudice and previously pending Claims 1, 69, 70, 72, 73 are currently amended. New Claims 87-107 have been introduced herein. The objection to Claim 13 is rendered moot by cancellation. The objection to the misnumbering of Claim 87 has been addressed by renumbering that Claim to 86 in accordance with 37 C.F.R. §1.126.

Support for Claim Amendments

Claim 1:

For "HCV subtype 1b":

"An HCV subtype Ib replicon was constructed which is similar to the replicon described in Lohmann et al., Science 235 : 110-113 (1999)." (Page 59, lines 6-8)

For such that the adaptive mutation results in a change in the NS5A amino acid sequence selected from the group consisting of Ser (1179) to Ile, Arg (1164) to Gly, Ala(1174) to Ser, Ser(1172) to Cys, and Ser(1172) to Pro of SEQ ID:

"Examples of these adaptive mutations are those that encode an amino acid sequence change selected from the group consisting of Ser (1179) to Ile, Arg (1164) to Gly, Ala (1174) to Ser, Ser (1172) to Cys, and Ser (1172) to Pro of SEQ ID NO : 3." (Page 13, lines 1-3).

For Claims 69, 70, 72, 73, 100, 101, 102 and 103:

For isolated host cell : "Additionally, the invention is directed to cells comprising the above expression vectors as well as host cells comprising any of the polynucleotides described above. The host cells are preferably mammalian cells, more preferably human cells." (Page 13, lines 29-32).

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Support for Claim Amendments (continued):

For Claims 69, 70, 72, 73, 100, 101, 102 and 103:

For a non-human transgenic organism : "Thus, the functional HCV variants described here, or parts thereof, can be used to create transgenic models relevant to HCV replication and pathogenesis. In one example, transgenic animals harboring the entire genome of an HCV variant can be created. Appropriate constructs for transgenic expression of the entire HCV variant genome in a transgenic mouse of the invention could include a nuclear promoter engineered to produce transcripts with the appropriate 5' terminus, the full-length HCV variant cDNA sequence, a cis-cleaving delta ribozyme [Ball, J.Virol. 66: 2335-2345 (1992); Pattnaik et al., Cell 69 : 1011-1020 (1992)] to produce an authentic 3' terminus, followed possibly by signals that promote proper nuclear processing and transport to the cytoplasm (where HCV RNA replication occurs)." (Page 43, lines 23-32)

For an isolated host cell engrafted in a host organism: "In one manifestation, the variants could be used to inoculate immunodeficient mice harboring human tissues capable of supporting HCV replication. An example of this art is the SCID : Hu mouse, where mice with a severe combined immunodeficiency are engrafted with various human (or chimpanzee) tissues, which could include, but are not limited to, fetal liver, adult liver, spleen, or peripheral blood mononuclear cells. Besides SCID mice, normal irradiated mice can serve as recipients for engraftment of human or chimpanzee tissues. These chimeric animals would then be substrates for HCV replication after either *ex vivo* or *in vivo* infection with defined virus-containing inocula." (Page 42, lines 19-26)

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Support for Claim Amendments (continued):

For Claims 87, 88, 89, 104, 105, 106, and 107 (New):

"In addition to the identified NS5A mutations, nucleotide substitutions were also noted in NS3 and NS4B; Clone II (SEQ ID NO : 9) contains substitutions at nt 3550 (NS3) and nt 4573 (NS4B) (Lys(584) to Glu, and Ser (925) to Gly of SEQ ID NO : 3, embodied in SEQ ID NO: 17), whereas nt 2060 (NS3) was mutated in Clone VI (Figure 7, corresponding to Gln (87) to Arg of SEQ ID NO : 3, embodied in SEQ ID NO: 15)." (Page 61, Line 35-37; Page 62, lines 1-3).

For Claim 90 (New):

"Other adaptive mutations include a deletion of at least a portion of the ISDR, and may comprise the entire ISDR. In a particular embodiment, the adaptive mutation comprises a deletion of nucleotides 5345 to 5485 of SEQ ID NO : 6." (Page 61, lines 3-6)"

For Claim 91 (New):

"In addition, G418-resistant colonies were observed after transfection of HeLa cells, a human epithelial cell line, with replicon RNA of clone I. Therefore, at least one of the mutations that was adaptive in Huh7 cells also allows the establishment of HCV replication in a non-hepatic cell line." (Page 62, Line 12-16) and "In one instance, a deletion of 47 amino acids (I ; Figure 7), encompassing the interferon sensitivity determining region (ISDR), was found." (Clone I identification; Page 61, lines 31-33).

For Claim 92 (New):

"The adaptive mutations can also cause the polynucleotide to have attenuated virulence, wherein the HCV is impaired in its ability to cause disease, establish chronic infections, trigger autoimmune responses, and transform cells." (Page 12, lines 28-31)

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For Claims 93-97 (New):

"In still other embodiments, the present invention is directed to an HCV variant that has (a) transfection efficiency greater than 0.01%, as determined by replication-dependent neomycin resistance, or (b) greater ability of initial colonies of cells transfected with the variant to survive subpassage than wild-type HCV genotype 1, subtype Ib." (Page 14, Lines 24-27) and "In preferred embodiments, the transfection efficiency is greater than 0.1%; in more preferred embodiments, greater than 1%; in still more preferred embodiments, greater than 5%. In the most preferred embodiments, the transfection efficiency is about 6%." (Page 14, Lines 31-34)

Claim Rejections 35 U.S.C. §101

Pending Claims 69, 70, 72, and 73 were rejected in the last Office Action as being directed toward non-statutory subject matter (i.e. a human). Applicant has adopted the Examiner's suggestion that the claims be amended to recite an isolated host cell or an isolated host cell engrafted in a host organism. Support for the claim language directed to isolated host cells engrafted in a host organism is found on page 42, lines 15-26 of the specification. In the currently amended Claim 69, the claim is further directed away from non-statutory subject matter by specifying a non-human transgenic organism. Support for claim language specifying non-human transgenic organisms can be found on page 43 lines 1-23. Taken together, these amendments clearly direct currently pending Claims 69, 70, 72, 73, 100, 101, 102 and 103 to statutory subject matter (i.e. isolated host cells or non-human transgenic organisms). Please note that the applicant will address the Examiner's 35 U.S.C. §112 rejections of pending Claims 69, 70, 72, and 73 in a following section of this response.

Claim Rejections 35 U.S.C.§112

Applicant notes that Claims 7,8,12,13, and 15-17 were rejected under various paragraphs of 35 USC 112. These rejections are now rendered moot by the

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cancellation of these claims. Applicant further notes that the cancellation of Claims 7,8,12,13, and 15-17 is without prejudice.

35 U.S.C.§112 Rejections: Enablement

Examiner rejected then pending Claims 1,3-8,9,12,13, 15-16, 29, 61, 62, 69, 70, 72, 73 and 87 under the first paragraph of 35 U.S.C.§112 for failure to enable adaptive mutations in any HCV sequence. However, the Examiner did acknowledge that the specification was enabling for adaptive mutations in an HCV subtype 1b sequence (Page 4, Paragraph 13 of the last Office Action). Applicant traverses this rejection by currently amending Claim 1 and all dependent claims to specifically recite "A polynucleotide comprising a non-naturally occurring HCV subtype 1b sequence...".

Examiner also rejected then pending Claims 69, 70, 72 and 73 under the first paragraph of 35 U.S.C.§112 for failure to enable gene therapy and transgenic animals. The Examiner will note that the arguments presented herein with regard to enablement of currently pending Claims 69, 70, 72 and 73 also apply to the enablement of new Claims 100, 101, 102, and 103. With regard to gene therapy, this rejection is rendered moot as the currently amended claims specify "a non-human transgenic organism".

With regard to production of non-human transgenic animals, the applicant maintains that the original disclosure and references found on pages 38, lines 6-35 and page 43, lines 1-22 of the application as originally filed would enable one skilled in the art to practice the invention as claimed without undue experimentation. First, in the field of transgenic organisms, both the level of the prior art and those skilled in that art was high at the filing date of May 2000. The high level of skill in the art is evidenced by the disclosed references on Page 43 of the specification that describe methods for production of transgenic animals dating back to 1985 (for example, see Hammer et al. Nature 315:680). The references cited by the applicant in the specification thus establish that there was "a high level of skill in the art at the time the application was

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filed", a key factor in considering the level of enablement needed to practice the invention (*In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

Secondly, the level of predictability involved in obtaining non-human transgenic organisms that express a given gene of interest is in fact much higher than asserted by the Examiner. While the Examiner's observation that the site of integration is unpredictable and influences transgene expression is true in the case of randomly integrated transgenes, this problem is routinely addressed by those skilled in the art by simply creating a large number of transgenic events and screening for those that express the transgene. This point is well illustrated by U.S. Patent 5,530,177, cited on Page 43 of the specification, that describes the injection of over 40 mouse embryos to obtain three (3) transgenic lines that expressed the transgene. The routine nature of that particular experiment conducted prior to 1993 is further evidenced by the U.S. Patent 5,530,177 disclosure that the experiments were executed at a University "service" facility that provides transgenic mice to multiple research groups on a regular basis (Column 12, Lines 31-35). The past and present existence of contract transgenic animal production facilities is not consistent with the notion that production of transgenic animals is an entirely unpredictable art. If that were the case, such contract service facilities would not be viable. Finally, the specification also cites the Joyner reference (Joyner, In Gene Targeting : A Practical Approach. The Practical Approach Series, Rickwood, D., and Hames, B. D., Eds., IRL Press: Oxford (1993); Page 43, lines 6 and 7) that discloses techniques for obtaining non-random, targeted transgene insertions with embryonic stem cells. As noted in the Houdebine reference cited by the Examiner, this procedure leads to "precise replacement of one gene by another" and that "the foreign gene introduced with this method will be well-expressed" (pages 278 and 279 of Houdebine, J. Biotechnology 34:269).

Thirdly, the level of guidance and direction provided by the Applicant also argues for enablement of claims directed to non-human transgenic organisms. As noted by the

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Examiner, clear guidance on the use of appropriate regulatory regions in expression vectors for transgenic hosts is critical. Numerous examples of both constitutive and tissue-specific promoters active in mammals are explicitly provided on pages 36, lines 4-35 of the specification. Additional guidance on use of mammalian expression vectors that incorporate selectable markers is also disclosed on page 38, lines 6-35. Further direction is provided in an explicit description of an HCV transgenic expression vector containing a nuclear promoter engineered to produce transcripts with a proper 5' terminus, a full-length variant HCV cDNA sequence, and ribozyme sequences to produce an authentic HCV 3' terminus (page 43, lines 24-33 of the specification).

The Applicant also argues that the production of transgenic organisms that are phenotypically normal does not need to be enabled in order to practice the invention as claimed. Indeed, one object of the instant application is to create transgenic animal models that do exhibit phenotypic or pathogenic effects to evaluate the efficacy of antiviral agents or to gain insight into mechanisms of HCV pathogenesis (page 43, lines 12-14, 23-24; page 44, lines 1-5). The use of transgenic animal models that are phenotypically compromised was clearly guided by the references to transgenic models of various viral and non-viral diseases cited by the Applicant (Weighart et al; Quon et al; Chisari; and WO 96/0927; page 43, lines 15-21). In contrast, enablement of transgenic organisms that are phenotypically normal is relevant to use of transgenic organisms for commercial production of pharmaceutical proteins as described in the Houdebine reference cited by the Examiner (Houdebine, J. Biotechnology 34:269). In that case, the object of creating the transgenic animals is to produce high levels of the gene of interest that are cost-competitive with other non-transgenic means of producing the pharmaceutical protein. For commercial production of pharmaceutical proteins in transgenic animals, phenotypic effects are clearly undesirable as they would increase production costs.

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Finally, it is also unlikely that transgenic organisms expressing HCV variant sequences would not be viable. First, the specification provides evidence that various cell lines can support HCV variant replication without loss of viability. Secondly, active HCV infections of the preferred primate host typically result in chronic disease rather than acute toxicity (page 2, lines 7-10). It is thus exceedingly unlikely that transgenically introduced HCV variant sequences would result in acute toxicity.

35 U.S.C.§112 Rejections: Written Description

In the previous Office Action, the Examiner rejected all then pending claims for failure to meet the written description requirement 35 U.S.C. §112, raising several distinct arguments pertaining to subsets of the claims. The Applicant has specifically addressed each of these arguments as follows.

The first argument concerned then pending Claims 7 and 8, which respectively recite polynucleotides capable of replication in non-hepatic and HeLa cells. In the Office Action, the Examiner did note that the Applicant identified a mutation that results in the ability of an HCV polynucleotide to replicate in HeLa cells. To address this argument, Claims 7 and 8 have been cancelled and replaced by the new Claim 91 that recites HCV polynucleotides capable of replication in a HeLa cell and is dependent on the new Claim 90 that recites the NS5A deletion mutation shown by the applicant to permit replication in HeLa cells. Support for both new Claim 90 and new Claim 91 is found on page 62, lines 4-16 of the specification as originally filed.

The second argument alleged that the specification as filed failed to provide adequate written description of the broad genus of adaptive mutations of the then pending Claim 1 and its dependent claims. This argument is traversed by the currently amended Claim 1 and new Claim 90 that respectively recite the four adaptive NS5A point mutations and the adaptive NS5A deletion mutation identified in the specification as originally filed by the applicant.

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35 U.S.C.§102(e) Rejections: Novelty

The current Office Action has maintained the rejection of Claims 1, 61, 62, 69, 70, 72, 73 and 87 under 35 U.S.C. §102(e) as being anticipated by U.S. Patent No. 6,630,343 to Bartenschlager (hereafter referred to as the '343 Patent). That patent has a filing date of March 31, 2000, and claims priority to DE 199 15 178, filed on April 3, 1999.

The Applicant maintains that the presently amended Claims 1, 61, 62, 69, 70, 72, 73 and 86 are no longer anticipated by the '343 Patent as they now specify adaptive mutations that were not disclosed in the '343 Patent. In withdrawing the 102e/103 rejection of Claims 14 and 17 in the previous Office Action, the Examiner notes that neither the '343 Patent nor the art provide any suggestion or guidance to modify the HCV NS5A sites indicated by Claim 14 or 17 (Page 16, lines 4-6). The same HCV NS5A site modifications of the previously pending Claim 14 are now specified in the currently amended Claim 1 and dependent Claims 61, 62, 69, 70, 72, 73 and 86. Since the '343 Patent fails to provide any suggestion or guidance leading to the HCV NS5A modifications claimed in this application, it necessarily follows that the '343 Patent fails to anticipate the instant claims of this application. Since the '343 Patent does not anticipate HCV NS5A deletion mutation listed in the previously pending Claim 17, this same argument also applies to new Claims 90-107 that specify the unanticipated HCV NS5A deletion mutation.

Given that the instant claims are not anticipated by the cited reference, Applicant respectively requests reconsideration and withdrawal of the rejection under 35 U.S.C. §102(e).

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35 U.S.C.§102(e)/103 Rejections: Obviousness

Previously presented Claims 3-6, 12, 13, 15, 16 and 29 were rejected under 35 U.S.C. §102(e) as anticipated by or, in the alternative, under 35 U.S.C. §103(a) as obvious over U.S. Patent No. 6,630,343 to Bartenschlager. The amended Claims 3-6 and 29 now incorporate the HCV NS5A modifications of Claim 14 that, as noted by the Examiner, are neither guided nor suggested by U.S. Patent No. 6,630,343 to Bartenschlager. The same arguments also apply to new Claims 90-107. Since new Claims 90-107 specify the same HCV NS5A deletion listed in the previously pending Claim 17, the '343 Patent also fails to guide or suggest this modification. The applicant consequently requests reconsideration and withdrawal of the rejection.

Claim 9 is rejected under 35 U.S.C. §103(a) as being unpatentable over the '343 Patent. As previously discussed, the inventions of the instant Claim 9 now specify NS5A mutations that find no guidance nor suggestion in the '343 Patent. This same argument also applies to new Claim 92. Therefore, applicant respectfully requests reconsideration and withdrawal of the rejection.

Conclusion:

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action, and as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, he is invited to telephone the undersigned at the number provided.

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Prompt and favorable consideration of this Amendment is respectfully requested.

Respectfully submitted,

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